

DIAGNOSTIC EVALUATION OF EXTRAPULMONARY TUBERCULOSIS: A COMPARATIVE STUDY OF FNAC, ZN STAINING, AND CB-NAAT TECHNIQUES

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Received: 19 Oct 2025, Revised and Accepted: 26 Nov 2025

ABSTRACT

Objective: To evaluate and compare the diagnostic efficacy of Fine Needle Aspiration Cytology (FNAC), Ziehl–Neelsen (ZN) staining, and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in the diagnosis of extrapulmonary tuberculosis (EPTB).

Methods: A prospective observational study was conducted on clinically suspected EPTB cases. FNAC smears were examined cytologically, ZN staining was performed for acid-fast bacilli, and CBNAAT was used for molecular detection of *Mycobacterium tuberculosis* and rifampicin resistance.

Results: FNAC showed high sensitivity and rapid diagnostic utility, especially in resource-limited settings. ZN staining demonstrated very high sensitivity and strong rule-out value, but lower specificity. CBNAAT exhibited the highest specificity and reliably identified rifampicin resistance, making it the most effective confirmatory test. Higher EPTB incidence was observed among females.

Conclusion: A tiered approach—initial screening with FNAC or ZN staining followed by CBNAAT confirmation—provides accurate, feasible, and cost-effective EPTB diagnosis.

Keywords: EPTB, FNAC, ZN staining, CBNAAT, Tuberculosis diagnosis

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INTRODUCTION

Extrapulmonary tuberculosis (EPTB) accounts for nearly 25% of the global TB burden, although pulmonary involvement continues to be the most common presentation [1]. Most EPTB cases lack active lung disease apart from tuberculous lymphadenitis. Because EPTB occurs in diverse anatomical sites such as lymph nodes, bones, and the genitourinary system, diagnosis is often challenging. Its nonspecific symptoms may mimic chronic inflammatory or neoplastic conditions, leading to delayed or missed diagnosis [2]. Lymphadenopathy and serous effusions constitute the most frequent clinical manifestations of EPTB.

Among the diagnostic tools, fine needle aspiration cytology (FNAC) is widely used as a minimally invasive, first-line procedure for evaluating suspected extrapulmonary lesions. FNAC allows cytological assessment for epithelioid granulomas, caseous necrosis, and other features suggestive of tuberculous lymphadenitis. However, morphological overlap with other granulomatous disorders necessitates confirmation by ancillary tests such as Ziehl–Neelsen (ZN) staining, cartridge-based nucleic acid amplification test (CBNAAT), or culture [3].

ZN staining remains a conventional, cost-effective method for detecting acid-fast bacilli, but shows reduced sensitivity in EPTB due to the typically low bacillary load. In contrast, CBNAAT, a rapid, real-time PCR assay, detects *Mycobacterial* DNA and identifies rifampicin resistance within two hours. In 2014, the WHO recommended CBNAAT as the preferred diagnostic method for EPTB specimens because of its superior sensitivity, specificity, and ability to detect drug resistance [4].

In 2021, there were 10.6 million TB cases and 1.6 million fatalities, up 3.6% from 2020's 10.1 million cases and 1.5 million deaths, according to the World Health Organization's (WHO) 2022 report. Fifty percent of HIV-confirmed cases are extrapulmonary TB, which makes about 15–20% of total TB cases [5].

This study compares FNAC, ZN staining, and CBNAAT to evaluate their diagnostic performance in EPTB. By analyzing their relative strengths and limitations, the study aims to determine the most

reliable diagnostic approach, thereby supporting improved clinical decision-making in regions where EPTB is widely prevalent.

MATERIALS AND METHODS

TB is the leading infectious killer in the world, killing 1.5 million people annually despite being a preventable and curable illness. *Mycobacterium tuberculosis* is latently present in one-fourth of the world's population [6]. This prospective observational study was conducted in the Department of Pathology, Hi-Tech Medical College and Hospital, Rourkela, Odisha. The study included both outpatient and inpatient cases referred for lymph node and superficial soft tissue FNAC. The study period extended from 1 September 2023 to 31 December 2024. A total of 202 samples were included, with the sample size calculated using OpenEpi (Version 3) software. Institutional Ethics Committee approval was obtained (IEC Ref. No. 13, ESICMCLB/IEC/2018-19).

Eligibility criteria

All clinically and cytologically suspected EPTB cases of all ages and both genders referred for FNAC were included. Patients with suspected cervical tubercular lymphadenopathy (18–65 y), individuals with a history of tuberculosis, and those currently on anti-tubercular therapy were also enrolled. Cases with inadequate aspirates, inaccessible deep-seated lesions requiring radiological guidance, fluid or sputum samples, and patients unwilling to provide consent were excluded.

Data collection

After obtaining informed consent, clinical history, demographic details, site of FNAC, and aspirate characteristics were recorded. All findings from FNAC, ZN staining, and CB-NAAT were entered into an Excel-based database.

Sample collection and processing

FNAC was performed using a 22-gauge needle and 10 ml syringe. Multiple smears were prepared: air-dried smears were stained with Giemsa, while alcohol-fixed smears were stained with Papanicolaou and Hematoxylin–Eosin stains. Cytological evaluation focused on epithelioid granulomas, caseous necrosis, and Langhans giant cells.

A dedicated smear was stained using the Ziehl-Neelsen method to detect acid-fast bacilli under oil immersion.

For CB-NAAT, a portion of the aspirate was processed using the Xpert MTB/RIF system following manufacturer instructions. The sample was mixed with reagent, incubated for 15 min, and 2 ml was loaded into the cartridge. Results were reported as MTB detected/not detected and rifampicin-sensitive/resistant.

Outcome measures

The study aimed to determine the sensitivity and specificity of FNAC, ZN staining, and CB-NAAT; assess diagnostic concordance; and evaluate feasibility and rapidity in diagnosing EPTB to support early initiation of anti-tubercular therapy.

RESULTS

A total of 202 patients were included in the study, comprising 37% males and 63% females, with a male-to-female ratio of 1:1.6. The noticeably higher number of female cases may reflect gender-based differences in disease susceptibility, healthcare-seeking behavior, or socio-economic influences affecting access to diagnostic facilities.

Age distribution

The highest number of cases was observed in the 21–30 y age group, accounting for 84 cases (41.58%), of which 68 (81%) were females. This was followed by the 31–40 y age group with 32 cases (15.84%), 41–50 y with 28 cases (13.86%), and 11–20 y with 20 cases (9.90%). The least affected age groups were 61–70 y (4 cases, 1.98%) and 81–90 y (2 cases, 0.99%).

Cytological and diagnostic findings

Among the 202 cases, tubercular lymphadenitis was the most common diagnosis, identified in 117 cases (57.92%). Other findings included tubercular lesions (14 cases, 6.93%), granulomatous lymphadenitis (22 cases, 10.89%), granulomatous lesions (8 cases, 3.96%), reactive lymphadenitis (11 cases, 5.45%), suppurative lymphadenitis (9 cases, 4.46%), suppurative lesions (15 cases, 7.43%), and metastatic carcinoma (6 cases, 2.97%).

A highly significant association was found between CBNAAT and ZN stain results ($p < 0.05$), indicating strong diagnostic concordance. The Chi-square test further demonstrated a significant association among FNAC, ZN staining, and CBNAAT, reaffirming their complementary diagnostic roles. Notably, 71.4% of CBNAAT-positive cases were females, contrasting with previous studies and suggesting a need to explore gender-specific influences.

Performance of diagnostic modalities

FNAC detected all 117 cases of tubercular lymphadenitis and 14 tubercular lesions, amounting to 131 positive cases (64.85%). It demonstrated a sensitivity of 90.09% and specificity of 81.32%, confirming its value as a cost-effective initial diagnostic tool.

ZN staining identified 131 positive cases, matching FNAC. Its Chi-square association with FNAC was significant ($p < 0.05$). It yielded the highest sensitivity (100%), though with lower specificity (83.53%), making it highly effective for ruling out tuberculosis yet prone to false positives.

CBNAAT detected 111 cases (54.95%), with a strong association with ZN staining ($p < 0.05$). It demonstrated the highest specificity (87.06%) and a sensitivity of 85.47%, making it the most reliable method for confirming TB while minimizing false positives.

Overall, FNAC and ZN staining provided high detection rates, whereas CBNAAT offered superior specificity, underscoring the diagnostic benefit of using all three modalities in combination for accurate detection of extra pulmonary tuberculosis.

DISCUSSION

Extra-pulmonary tuberculosis (EPTB) continues to pose a significant global health challenge, particularly in developing countries, where delayed diagnosis contributes to increased morbidity and mortality. The microscopic diagnosis of pulmonary TB is essential in

developing countries because it is inexpensive, rapid and sensitive, but the sensitivity is limited to 20–43% for EPTB patients [7]. The present study evaluated and compared the diagnostic performance of Fine Needle Aspiration Cytology (FNAC), Ziehl-Neelsen (ZN) staining, and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in identifying EPTB, while also analyzing demographic variations and gender-specific trends.

In this study of 202 patients, a higher proportion of cases were females (63%) compared to males (37%). This contrasts with multiple earlier reports, where TB prevalence was consistently higher among males. The gender shift noted in the present study may be attributed to healthcare-seeking behavior, with women more likely to access medical care earlier, as well as sociocultural influences that may delay men from seeking timely evaluation. Hormonal influences, including the immunomodulatory effects of estrogen, may also contribute to differing disease susceptibility. Further research is needed to explore these gender-specific determinants.

The age distribution showed that the majority of cases occurred in young adults aged 21–30 y (41.6%), followed by those aged 31–40 y (15.8%). This aligns with global epidemiological data indicating that young adults constitute a high-risk group, possibly due to increased exposure, greater social mobility, and immunological factors. The elderly population exhibited the lowest prevalence, which may reflect underreporting, reduced healthcare utilization, or a genuinely lower burden of EPTB in advanced age.

Comparative evaluation of diagnostic modalities revealed significant insights. FNAC identified 131 positive cases (64.85%), demonstrating high sensitivity (90.09%) and specificity (81.32%). These findings reaffirm FNAC as a dependable first-line diagnostic method, particularly in resource-limited settings where rapid, minimally invasive, and cost-effective techniques are essential. However, FNAC may produce false positives, especially in granulomatous conditions unrelated to tuberculosis.

ZN staining also detected 131 cases, providing the highest sensitivity (100%) but moderate specificity (83.53%). The excellent sensitivity highlights its reliability for screening and ruling out tuberculosis. Nevertheless, its inability to differentiate *Mycobacterium tuberculosis* from non-tuberculous mycobacteria and its reduced effectiveness in paucibacillary samples limit its standalone diagnostic value.

CBNAAT detected 111 positive cases (54.95%) and demonstrated the highest specificity (87.06%) with a sensitivity of 85.47%. The strong statistical association between CBNAAT and AFB results ($p < 0.05$) underscores its diagnostic strength. CBNAAT's ability to detect Mycobacterial DNA in paucibacillary conditions, along with simultaneous rifampicin resistance testing, offers significant advantages over conventional techniques. Although its sensitivity was marginally lower than ZN staining, its superior specificity makes CBNAAT particularly suitable for confirmatory diagnosis and guiding treatment decisions.

A comparison with previous studies further highlights gender-related variability. While Rao CM *et al.* (2018) reported a male predominance in male (54.6%) than female (45.4%) [8] and Sahukar *et al.* (2022) reported a males (48.8%) and females (51.2%) [9]. The present study found 71.4% of CBNAAT-positive cases among females. This discrepancy may reflect regional variation in TB epidemiology, differential healthcare access, and occupational or environmental exposures.

Given the limitations of individual diagnostic methods, the study supports an integrated diagnostic approach. FNAC serves as an effective initial tool, ZN staining allows rapid screening, and CBNAAT offers confirmatory precision with drug-resistance detection. This combined strategy enhances diagnostic accuracy, facilitates early treatment initiation, and improves patient outcomes. Morbidity, higher mortality and disease sequelae are the results of delayed diagnosis [10].

The study has certain limitations, including its single-center design, absence of culture confirmation (the gold standard), limited sample

size, and lack of rifampicin-resistant TB analysis. Future multicenter studies incorporating culture and comprehensive drug-resistance profiling are recommended to validate these findings and strengthen diagnostic protocols.

CONCLUSION

Extrapulmonary tuberculosis (EPTB) requires timely and accurate diagnosis to reduce morbidity. This study demonstrates that FNAC, ZN staining, and CBNAAT each play a complementary role in diagnosing EPTB. FNAC remains a sensitive, rapid, and cost-effective first-line tool suitable for resource-limited settings. ZN staining provides high sensitivity and an excellent rule-out value, though with lower specificity. CBNAAT, with its superior specificity and ability to detect rifampicin resistance, serves as the most reliable confirmatory test. A tiered diagnostic approach—FNAC or ZN staining for initial screening followed by CBNAAT confirmation—offers optimal accuracy and feasibility. The higher female prevalence observed highlights the need for gender-focused TB awareness and improved diagnostic access.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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